FIG. 4. Effect of O_2 on the motility of rainbow trout spermatozoa. (a), Relative percentage of dissolved oxygen (○) and motility of spermatozoa (△) in 100 mM NaCl when N_2 was bubbled for indicated time. (b), Percentage of dissolved oxygen in 100 mM NaCl containing $Na_2S_2O_4$ (○). Percentage (△) or swimming velocity (▲) of motile spermatozoa. Vertical bars represent Means±S.E. in 20–25 spermatozoa.
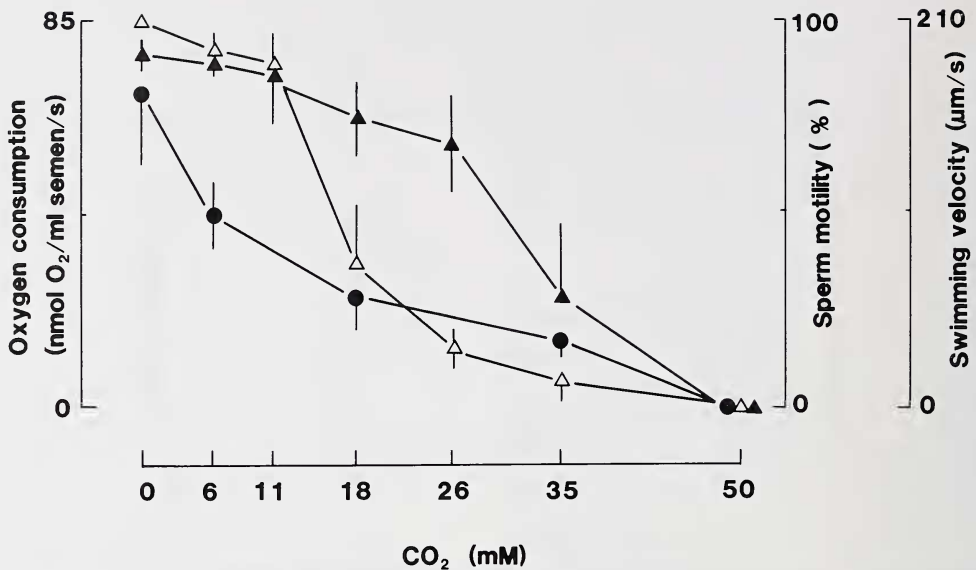
FIG. 5. Effect of CO_2 on the oxygen consumption and motility of rainbow trout spermatozoa. Percentage ($\triangle$) or swimming velocity ($\blacktriangle$) of motile spermatozoa. Oxygen consumption of spermatozoa ($\bullet$). Vertical bars represent Means $\pm$ S.E. in 3 experiments.

[5]. Although our recent studies have demonstrated the detailed mechanism of K^+ dependent initiation process of trout sperm motility [1], the target site of K^+ has been left somewhat unclear. In this paper, it was shown that the oxygen consumption of sperm, of which motility was suppressed by K^+, was almost similar to that of motile spermatozoa in the K^+ free medium (Fig. 1). This suggested that K^+ does not suppress mitochondrial respiration but do flagellar movement. Furthermore, target of K^+ may be plasma membrane of sperm flagella since flagella of which plasma membrane was removed are able to beat in the presence of K^+ [6].

It has been reported that immotile trout spermatozoa retain a high concentration of ATP, while a rapid decrease of ATP level occurs within very short period when spermatozoa initiate motility [8]. This phenomenon might be correlated with the short term oxygen consumption of trout spermatozoa at the initiation of motility which occurs within a very short period. The short term oxygen consumption of trout sperm increased with increase of dilution ratio (Fig. 2). From the result, it seems to be considered that gradual activation of mitochondrial function occurs at natural spawning

when spermatozoa are released and gradually diluted in water. In the process, some changes of volatile factor in the circumstance of sperm may possibly relate to the initiation of energy supply and sperm motility. Thus there is some room for further examining the correlation between sperm respiration and initiation of motility.

NaN_3 and KCN, inhibitors of respiratory chain, or CCCP, an uncoupler of oxidative phosphorylation, suppressed sperm motility (Fig. 3), suggesting that sperm motility seems to be restricted by the energy supplying systems. These results confirmed our preliminary data [9].

Many investigators reported that sperm respiration and motility are affected considerably by O_2 and CO_2 (see [1]). Rothschild [2] reported that sea urchin spermatozoa in a gas-tight chamber were immotile when N_2 was introduced, however spermatozoa became motile when O_2 was introduced into the chamber. However, the opposite conclusion was proposed by Johnson et al. [4]. In rainbow trout, as shown in Figure 4, spermatozoa could initiate and maintain motility in O_2 deficient medium, even in a completely anaerobic medium obtained by the addition of $Na_2S_2O_4$. This result suggests that O_2 is not a limiting factor for sperm

motility in this species. A change from anaerobic to aerobic condition, which might occur at natural spawning, may not affect sperm motility.

CO_2 is reported as a suppressor of sperm motility in many animals [1]; for example, motility and respiration of sea urchin spermatozoa are reversibly suppressed by CO_2 [4]. In rainbow trout, CO_2 influenced inhibitorily to the sperm respiration and motility (Fig. 5). Thus, CO_2 seems to be an attractive candidate as the factor for suppressing the sperm respiration and motility in the semen in reproductive organ in which CO_2 is present [10].

In conclusion, although there are some doubts whether volatile factor(s) physiologically restricts the initiation of trout sperm motility, it is attractive to predict that a volatile factor dependent system at mitochondria may contribute to the initiation of trout sperm motility, independently of the established K^+ dependent initiation mechanism at flagella.

ACKNOWLEDGMENTS

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Excitatory and Inhibitory Junction Potentials Recorded from the Red Muscle of Marine Teleost, Puffer Fish

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ABSTRACT—Junction potentials were recorded from the red muscle of two species of the marine teleost, puffer fish, *Takifugu poecilonotus* and *T. rubripes*. Three types of potential change, the excitatory junction potential (ejp), the inhibitory junction potential (ijp) and the diphasic junction potential composed of ejp and ijp, were elicited by the nerve stimulation, and the miniature excitatory junction potential (mejp) and the miniature inhibitory junction potential (mijp) were observed in the resting muscle. Thus, this muscle received the innervations from both excitatory and inhibitory nerves and the excitatory innervations were much more abundant than the inhibitory ones. Nicotinic antagonist of acetylcholine (ACh), *d*-tubocurarine (*d*-TC), suppressed ejp, ijp, mejp and mijp and anticholinesterase, neostigmine, augmented them, while muscarinic antagonist of ACh, atropine, did not affect them. The results suggested that the excitatory and the inhibitory neuromuscular transmissions of this muscle were cholinergic and the nature of the receptors was nicotinic. The present observations obtained in the marine teleost were almost the same as those reported in the freshwater teleost, silver carp.

INTRODUCTION

In the vertebrate skeletal muscle, it is well known that there are two muscle types, referred as fast and slow, phasic and tonic or white and red, respectively. As reviewed by Hess [1] and Morgan and Proske [2], the white muscle produces the action potential in response to the nerve stimulation, whereas the red muscle does not initiate the spike but responds with non-propagating junction potential in various kinds of vertebrate skeletal muscle. Similar observations were obtained in the white and red muscle of freshwater teleost [3, 4]. The red muscle of a silver carp was found to elicit the excitatory junction potential (ejp) by the nerve stimulation and to generate the miniature excitatory junction potential (mejp) in the resting muscle [4]. Recently, it was reported in the same nerve-muscle preparation that not only ejp but also the inhibitory junction potential (ijp) and the diphasic junction potential composed of ejp and ijp were

elicited by the nerve stimulation and that the miniature inhibitory junction potential (mijp) as well as mejp could be recorded from the resting muscle [6]. This observation was the first demonstration that ijp and mijp were recorded in the vertebrate skeletal muscle.

The experiment reported below was carried out in order to evaluate if the inhibitory innervation was present in red muscle of marine teleost and to compare the innervation pattern between the red muscles of freshwater and marine teleost fishes.

MATERIALS AND METHODS

Two species of puffer fish, *Takifugu poecilonotus* and *Takifugu rubripes*, 12–27 cm in body length, were used. Because significant differences in the results were not found between two species, the results obtained using *T. poecilonotus* were presented in this paper. Fishes were purchased from a fishery and were kept in the natural sea water saturated with air up to about a month. The nerve-muscle preparation was dissected from the red muscle of both sides of pectoral fin which was

innervated by the spinal motor nerves, Th. 1 and Th. 2, as described previously [5]. The preparation was placed in a chamber of 5 ml in volume and was perfused at a constant flow rate of 5 ml/min with artificial sea water (ASW) of the following composition (mM); NaCl 462, KCl 9.4, $CaCl_2$ 10.8, $MgCl_2$ 48.2 and $NaHCO_3$ 6.0 (pH 8.0). The methods of recording of the electrical responses and the nerve stimulation were the same as those in the previous study [6]. The following drugs were used; d-tubocurarine (d-TC, Sigma), neostigmine methylsulfate (Sigma) and atropine sulfate (Nakarai Chemicals). The effects of these drugs dissolved in ASW were tested by the bath application. The experiment was carried out at room temperature (18–24°C).

RESULTS

The resting potentials of the red muscle fiber were -70 ± 1.1 mV (mean$\pm$SE, n$=$25) in *Takifugu poecilonotus* and -69.5 ± 0.8 mV (mean$\pm$SE, n$=$25) in *T. rubripes*. The significant difference in the resting potential was not noted between the freshwater and the marine teleosts, the corresponding value of silver carp being -73.1 mV [5].

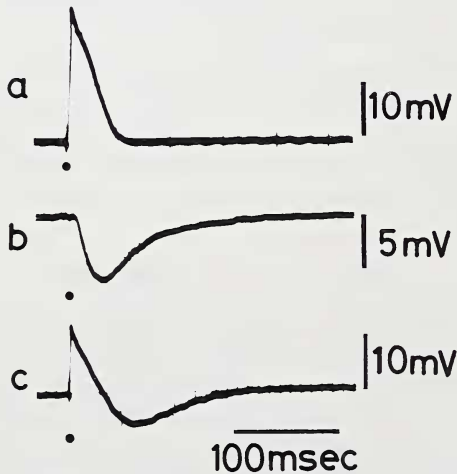Figure 1 shows the typical response to the single nerve stimulation recorded from the red muscle

Fig. 1. Three types of response elicited by single nerve stimulation. a; ejp, b; ijp, c; diphasic junction potential. Records taken from different muscle fibers. Dots in this and Fig. 3 indicate nerve stimulation.

fiber of *T. poecilonotus*. These potentials were ejp (a), ijp (b) and diphasic junction potential (c). The amplitude and the duration of three types of the response were different from fiber to fiber examined. In the diphasic junction potential consisted of ejp and ijp, ejp consistently proceeded ijp.

The amplitudes of ejps and ijps were 5.4 ± 0.6 mV (mean$\pm$SE, n$=$20) and 2.6 ± 2.0 mV (mean$\pm$SE, n$=$20) respectively, and the durations of ejp and ijp were 30.7 ± 3.7 msec (mean$\pm$SE, n$=$20) and 130.0 ± 14.2 msec (mean$\pm$SE, n$=$20) respectively. The amplitudes of depolarizing phase and hyperpolarizing phase of diphasic junction potentials were 6.7 ± 0.9 mV (mean$\pm$SE, n$=$20) and 2.2 ± 0.4 mV (mean$\pm$SE, n$=$20) respectively, and the duration of depolarizing phase and hyperpolarizing phase of diphasic junction potentials were 18.4 ± 2.3 msec (mean$\pm$SE, n$=$20) and 115.6 ± 14.9 msec (mean$\pm$SE, n$=$20) respectively. Thus, the amplitude of ejp exceeded that of ijp and the duration of ejp was shorter than that of ijp. This was the case in depolarizing phase and hyperpolarizing phase of diphasic junction potential.

In the resting muscle, the miniature junction potentials (mjps) were generated spontaneously. Figure 2 showed the sample records in which mejp (a), mijp (b) and both mejp and mijp (c) were observed in three different muscle fibers. An example generating only mijp was rare and was observed in only one fiber throughout the present experiment (b). It was noticed that mejps having two different, fast and slow, rise times were recorded from the same muscle fibers (a and c). From the observations presented in Figures 1 and 2, it was suggested that the red muscle of puffer fish might receive the double innervations from both the excitatory and the inhibitory nerves and the excitatory innervation might be distributed along the muscle fiber multiply.

Table 1 shows the number and the percentage, of the junction potentials (A) and the miniature junction potentials (B), which were recorded from the muscle fibers inserted with the microelectrode arbitrarily. Out of 425 muscle fibers examined, the percentages of ejp, ijp and diphasic junction potential were 56%, 16% and 28% respectively (A). Out of 120 muscle fibers, the percentages of
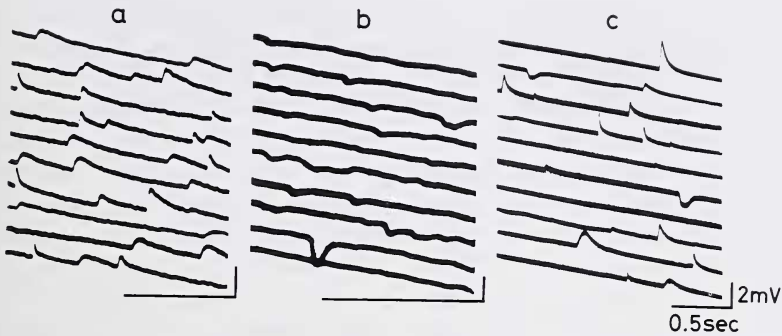
FIG. 2. Sample records of mejp (a), mijp (b) and both mejp and mijp (c) recorded continuously from different muscle fibers.

TABLE 1. Number (percentage) of junction potentials (jp) recorded from 425 muscle fibers (A) and of miniature junction potentials (mjp) recorded from 120 muscle fibers (B) of *T. poecilonotus*

(A) Number (%) of jp		(B) Number (%) of mjp	
ejp	238 (56)	mejp	103 (86)
ijp	68 (16)	mijp	1 (1)
diphasic jp	119 (28)	mejp and mijp	16 (13)
total	425	total	120

the fibers generating mejp, mijp and both mejp and mijp were 86%, 1% and 13% respectively (B). These percentages might roughly reflect the distribution of the excitatory and the inhibitory in-

FIG. 3. Effects of *d*-TC and neostigmine on diphasic junction potential. a1 and b1; control. a2; 15 min after 10^{-6} M *d*-TC. b2; 15 min after 10^{-6} M neostigmine. a and b; records from different muscle fibers. Partial recovery 60 min after washing (not shown).

nervations of this muscle and might suggest that the excitatory innervations are more abundant than the inhibitory ones.

To investigate the nature of the neurotransmitter and the receptor mediating the neuromuscular transmission of this muscle, the effects of *d*-TC and neostigmine on the diphasic junction potential were examined. As shown in Figure 3, diphasic junction potential was almost eliminated by 10^{-6} M *d*-TC (a2) and was augmented by 10^{-6} M neostigmine (b2). Similar effects of *d*-TC and neostigmine were observed on ejp, ijp and mijp. Atropine (10^{-6} M), the muscarinic antagonist of ACh receptor, had no appreciable effects on all types of junction potentials. The results indicate that the transmission is cholinergic at the neuromuscular junction of this muscle and the nature of the receptor is nicotinic.
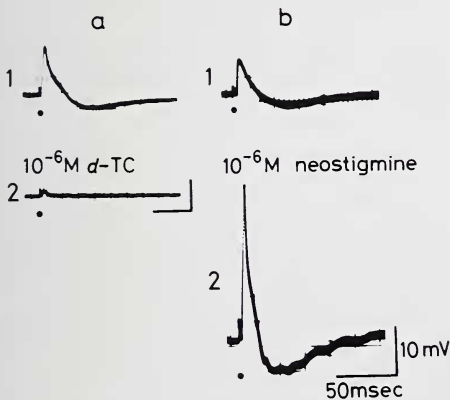
DISCUSSION

It was reported in the previous paper that depolarizing ejp, hyperpolarizing ijp and diphasic